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     (FILE 'HOME' ENTERED AT 14:16:21 ON 17 OCT 2002)

     INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
     BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO,
CABA,
     CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
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               SEA POLYSIALYLTRANSFERASE
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                 2   FILE AQUASCI
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                 2   FILE BIOTECHDS
                57   FILE BIOTECHNO
                 1   FILE CABA
                18   FILE CANCERLIT
                87   FILE CAPLUS
                 3   FILE CONFSCI
                 5   FILE DGENE
                 2   FILE EMBAL
                68   FILE EMBASE
                65   FILE ESBIOBASE
                 4   FILE FEDRIP
                38   FILE GENBANK
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                68   FILE MEDLINE
                 1   FILE OCEAN
                15   FILE PASCAL
                 1   FILE PROMT
               106   FILE SCISEARCH
                19   FILE TOXCENTER
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                 1   FILE WPIDS
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L1              QUE POLYSIALYLTRANSFERASE
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     FILE 'SCISEARCH, BIOSIS, CAPLUS, MEDLINE, EMBASE, ESBIOBASE, BIOTECHNO'
     ENTERED AT 14:17:48 ON 17 OCT 2002
L2            42 S L1 (S) K92
L3             0 S L2 AND (CDNA OR CLONE)
L4            11 DUP REM L2 (31 DUPLICATES REMOVED)
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=> d l4 ibib ab 1-11

L4 ANSWER 1 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 1
ACCESSION NUMBER: 2001:646142 SCISEARCH
THE GENUINE ARTICLE: 461DH
TITLE: Elongation of alternating alpha 2,8/2,9 polysialic acid by
the Escherichia coli **K92** **polysialyltransferase**
AUTHOR: McGowen M M; Vionnet J; Vann W F (Reprint)
CORPORATE SOURCE: Ctr Biol Evaluat & Res, Lab Bacterial Toxins, Div Bacterial Parasit & Allergen Prod, 8800 Rockville Pike, Bethesda, MD 20892 USA (Reprint); Ctr Biol Evaluat & Res, Lab Bacterial Toxins, Div Bacterial Parasit & Allergen Prod, Bethesda, MD 20892 USA
COUNTRY OF AUTHOR: USA
SOURCE: GLYCOBIOLOGY, (AUG 2001) Vol. 11, No. 8, pp. 613-620. Publisher: OXFORD UNIV PRESS INC, JOURNALS DEPT, 2001 EVANS RD, CARY, NC 27513 USA.
ISSN: 0959-6658.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 21
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have chosen E. coli **K92**, which produces the alternating structure alpha (2-8)neuNAc alpha (2-9)neuNAc as a model system for studying bacterial polysaccharide biosynthesis. We have shown that the **polysialyltransferase** encoded by the **K92** neuS gene can synthesize both alpha (2-8) and alpha (2-9) neuNAc linkages in vivo by C-13-nuclear magnetic resonance analysis of polysaccharide isolated from a heterologous strain containing the **K92** neuS gene. The **K92 polysialyltransferase** is associated with the membrane in lysates of cells harboring the neuS gene in expression vectors. Although the enzyme can transfer sialic acid to the nonreducing end of oligosaccharides with either linkage, it is unable to initiate chain synthesis without exogenously added polysialic acid. Thus, the **polysialyltransferase** encoded by neuS is not sufficient for de novo synthesis of polysaccharide but requires another membrane component for initiation. The acceptor specificity of this **polysialyltransferase** was studied using sialic acid oligosaccharides of various structures as exogenous acceptors. The enzyme can transfer to the nonreducing end of all bacteria polysialic acids, but has a definite preference for alpha (2-8) acceptors. Gangliosides containing neuNAc oc(2-8)neuNAc are elongated, whereas monsialylated gangliosides are not. Disialylgangliosides are better acceptors than short oligosaccharides, suggesting a lipid-linked oligosaccharide may be preferred in the elongation reaction. These studies show that the **K92 polysialyltransferase** catalyzes an elongation reaction that involves transfer of sialic acid from CMP-sialic acid to the nonreducing end of two different acceptor substrates.

L4 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:133873 CAPLUS
DOCUMENT NUMBER: 132:162041
TITLE: Escherichia coli strain **K92** gene neuS

.alpha.2,8/2,9-**polysialyltransferase**, its recombinant production, purification and activity

INVENTOR(S): Wong, Chi-Huey; Shen, Gwo-Jenn; Datta, Arun
PATENT ASSIGNEE(S): Scripps Research Institute, USA
SOURCE: PCT Int. Appl., 33 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000009736	A1	20000224	WO 1999-US18154	19990810
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9954758	A1	20000306	AU 1999-54758	19990810
EP 1105515	A1	20010613	EP 1999-941028	19990810
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				

PRIORITY APPLN. INFO.: US 1998-96003P P 19980810
WO 1999-US18154 W 19990810

AB The invention presents the recombinant prodn. of gene neuS .alpha.2,8/2,9-**polysialyltransferase** from Escherichia coli **K92**. The invention provides plasmid vectors contg. the neuS gene, host cells transformed with said vectors, and processes involved in obtaining the purified recombinant .alpha.2,8/2,9-polysialyltransferase. The invention also provides methods used to show that E. coli **K92** .alpha.2,8/2,9-**polysialyltransferase** is a functional enzyme able to convert a substrate to product. The invention specifically demonstrated PCR-based cloning of the E. coli neuS gene. A 1.2-kb PCR fragment was subcloned in pRSET vector and the protein was expressed in the BL21(DE3) strain of E. coli with a hexameric histidine at its N-terminal end. Western blotting using anti-histidine antibody showed the presence of a band that migrated at about 47.5 kD on both reducing and non-reducing SDS-PAGE, indicating a monomeric enzyme. Among the carbohydrate acceptors tested, N-acetylneuraminic acid and the gangliosides GD3 and GQ1b were preferred substrates. The cell-free enzyme reaction products obtained were characterized by NMR and mass spectrometry, which indicated the presence of both .alpha.2,9- and .alpha.2,8-linked polysialyl structure. The K92 neuS gene was used to transform the K1 strain of E. coli, the capsule of which contains only (-8-NeuAc.alpha.2-) linkages. Anal. of the polysaccharides isolated from these transformed cells is consistent with the presence of both (-8-NeuAc.alpha.2-) and (-9-NeuAc.alpha.2-) linkages, thus supporting that E. coli K92 catalyzes the synthesis of polysialic acid with .alpha.2,9- and .alpha.2,8- linkages.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 2
ACCESSION NUMBER: 1999:943586 SCISEARCH
THE GENUINE ARTICLE: 260XW
TITLE: Expression of alpha 2,8/2,9-**polysialyltransferase**

from Escherichia coli K92 - Characterization of t[redacted]enzyme and its reaction product[redacted]

AUTHOR: S[redacted] G J; Datta A K; Izumi M; Koeller K M; Wong C H (Reprint)

CORPORATE SOURCE: SCRIPPS CLIN & RES INST, DEPT CHEM, 10666 N TORREY PINES RD, BCC 338, SAN DIEGO, CA 92037 (Reprint); SCRIPPS CLIN & RES INST, DEPT CHEM, LA JOLLA, CA 92037; SCRIPPS CLIN & RES INST, SKAGGS INST CHEM BIOL, LA JOLLA, CA 92037

COUNTRY OF AUTHOR: USA

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (3 DEC 1999) Vol. 274, No. 49, pp. 35139-35146.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.

DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 41

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The capsular polysaccharide of Escherichia coil K92 contains alternating -8-NeuAc alpha 2- and -9-NeuAc alpha 2- linkages. The enzyme catalyzing this polymerizing reaction has been cloned from the genomic DNA of E. coil K92. The 1.2-kilobase polymerase chain reaction fragment was subcloned in pRSET vector and the protein was expressed in the BL21(DE3) strain of E. coli with a hexameric histidine at its N-terminal end, The enzyme was isolated in the supernatant after lysis of the cells and fractionated by ultracentrifugation. Western blotting using anti-histidine antibody showed the presence of a band that migrated at about 47.5 kDa on both reducing and nonreducing SDS-polyacrylamide gel electrophoresis, indicating a monomeric enzyme. Among the carbohydrate accepters tested, N-acetylneuraminic acid and the gangliosides G(D3) and G(Q1b) were preferred substrates. The cell-free enzyme reaction products obtained were characterized by NMR and mass spectrometry, which indicated the presence of both alpha 2,9- and alpha 2,8-linked polysialyl structure. The K92 neuS gene was used to transform the K1 strain of E. coil, the capsule of which contains only -8-NeuAc alpha 2- linkages. Analysis of the polysaccharides isolated from these transformed cells is consistent with the presence of both -8-NeuAc alpha 2- and -9-NeuAc alpha 2- linkages. Our results suggest that the neuS gene product of E. coil K92 catalyzes the synthesis of polysialic acid with alpha 2,9- and alpha 2,8-linkages in vitro and in vivo.

L4 ANSWER 4 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 3

ACCESSION NUMBER: 1999:149430 SCISEARCH
THE GENUINE ARTICLE: 166KQ

TITLE: Haemophilus ducreyi produces a novel sialyltransferase - Identification of the sialyltransferase gene and construction of mutants deficient in the production of the sialic acid-containing glycoform of the lipooligosaccharide

AUTHOR: Bozue J A; Tullius M V; Wang J; Gibson B W; Munson R S (Reprint)

CORPORATE SOURCE: CHILDRENS HOSP RES FDN, 700 CHILDRENS DR, ROOM W402, COLUMBUS, OH 43205 (Reprint); CHILDRENS HOSP RES FDN, COLUMBUS, OH 43205; OHIO STATE UNIV, DEPT PEDIAT, COLUMBUS, OH 43205; OHIO STATE UNIV, DEPT MED MICROBIOL, COLUMBUS, OH 43205; UNIV CALIF SAN FRANCISCO, DEPT PHARMACEUT CHEM, SAN FRANCISCO, CA 94143

COUNTRY OF AUTHOR: USA

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (12 FEB 1999) Vol. 274, No. 7, pp. 4106-4114.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 76

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Haemophilus ducreyi, the cause of the sexually transmitted disease chancroid produces a lipooligosaccharide (LOS) containing a terminal sialyl N-acetyllactosamine trisaccharide, Previously, we reported the identification and characterization of the N-acetylneuraminic acid cytidylsynthetase gene (neuA), Forty-nine base pairs downstream of the synthetase gene is an open reading frame (ORF) encoding a protein with a predicted molecular weight of 34,646, This protein has weak homology to the **polysialyltransferase** of Escherichia colt **K92**, Downstream of this ORF is the gene encoding the H, ducreyi homologue of the Salmonella typhimurium rmlB gene. Mutations were constructed in the neuA gene and the gene encoding the second ORF by insertion of an Omega kanamycin cassette, and isogenic strains were constructed. LOS was isolated from each strain and characterized by SDS-polyacrylamide gel electrophoresis, carbohydrate, and mass spectrometric analysis, LOS isolated from strains containing a mutation in neuA or in the second ORF, designated Ist, lacked the sialic acid-containing glycoform, Complementation studies were performed, The neuA gene and the ist gene were each cloned into the shuttle vector pLS88 after polymerase chain reaction amplification. Complementation of the mutation in the ist gene was observed, but we were unable to complement the neuA mutation, Since it is possible that transcription of the neuA gene and the Ist gene were coupled, we constructed a nonpolar mutation in the neuA gene, in this construct, the neuA mutation was complemented, suggesting transcriptional coupling of the neuA gene and the ist gene, Sialyltransferase activity was detected by incorporation of C-14-labeled NeuAc from CMP-NeuAc into trichloroacetic acid-precipitable material when the Ist gene was overexpressed in the nonpolar neuA mutant. We conclude that the Ist gene encodes the H, ducreyi sialyltransferase, Since the Ist gene product has little, if any, structural relationship to other sialyltransferases, this protein represents a new class of sialyltransferase.

L4 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:539790 CAPLUS
TITLE: Biosynthesis of polysialic acid as capsular polysaccharides in .
AUTHOR(S): McGowen, Margaret M.; Vionnet, Justine A.; Scates, Bradley A.; Vann, Willie F.
CORPORATE SOURCE: Center for Biologics Evaluation and Research, Bethesda, MD, 20892, USA
SOURCE: Book of Abstracts, 218th ACS National Meeting, New Orleans, Aug. 22-26 (1999), CARB-056. American Chemical Society: Washington, D. C.
CODEN: 67ZJA5
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English

AB Polysialic acids are synthesized by pathogenic bacteria as capsular polysaccharides. These polymers have been implicated in the virulence of some strains of Escherichia coli which cause neonatal meningitis and urinary tract infections. There has been significant progress in identifying the gene necessary for capsular polysacccharide biosynthesis in gram neg. bacteria. Much of the enzymol. of polysialic acid capsular polysaccharide synthesis has been done with the a-(2-8)polysialyltransferase complex of E.coli K1. Bacteria contg. DNA fragments encoding several capsule related genes have been used as a

source of enzyme activity. As a model system for investigating the mechanism of capsular glycosyltransferases we have chosen to investigate the **K92** a(2-8)(2-9) **polysialyltransferase** in a genetic background lacking other capsule related genes. The K92 PST requires an exogenously added acceptor when assayed in this genetic background. The **K92 polysialyltransferase** does not require neuE gene product for activity. The effect of acceptor repeat unit structure, chain lenght on elongation activity was detd. by the addn. of other sialic acid polymers, oligosialic acid, and gangliosides.

L4 ANSWER 6 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 1998:810804 SCISEARCH
THE GENUINE ARTICLE: 130CC
TITLE: Expression of the Escherichia coli **K92 polysialyltransferase**
AUTHOR: Vionnet J A (Reprint); McGowen M M; Scates B A; Vann W F
CORPORATE SOURCE: US FDA, CTR BIOL EVALUAT & RES, BETHESDA, MD
COUNTRY OF AUTHOR: USA
SOURCE: GLYCOBIOLOGY, (NOV 1998) Vol. 8, No. 11, pp. 79-79. Publisher: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.
ISSN: 0959-6658.
DOCUMENT TYPE: Conference; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 0

L4 ANSWER 7 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 4
ACCESSION NUMBER: 95:341028 SCISEARCH
THE GENUINE ARTICLE: QX543
TITLE: A FILTER ASSAY FOR POLYSIALYLTRANSFERASE
AUTHOR: VANN W F (Reprint)
CORPORATE SOURCE: US FDA, CTR BIOL EVALUAT & RES, BACTERIAL POLYSACCHARIDES LAB, 8800 ROCKVILLE PIKE, BETHESDA, MD, 20892 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: FEMS MICROBIOLOGY LETTERS, (01 MAY 1995) Vol. 128, No. 2, pp. 163-166.
ISSN: 0378-1097.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 19

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Polysialic acids occur as capsular polysaccharides of several pathogenic bacteria. An understanding of how **polysialyltransferase** functions in the synthesis of polysialic acid will require enzyme purification and characterization in concert with genetic analysis. A rapid filter assay has been developed for bacterial **polysialyltransferase** suitable for enzyme purification. The filter assay and the currently used paper chromatography methods are equivalent in parallel experiments. The Escherichia coli **K92 polysialyltransferase** exhibited the same pH and temperature optima, Mg2+ dependence and acceptor specificity in both assays. [C-14]Sialic acid bound in filter assays correlates with polymer formed by gel filtration. Specificity may be increased by the addition of exogenous accepters.

L4 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:577338 CAPLUS
DOCUMENT NUMBER: 119:177338
TITLE: Mechanisms of polysialic acid assembly in Escherichia coli K1: A paradigm for microbes and mammals
AUTHOR(S): Vimr, Eric R.; Steenbergen, Susan M.
CORPORATE SOURCE: Coll. Vet. Med., Univ. Illinois, Urbana, IL, 61801,

USA
SOURCE: Polysialic Acid (1993), 73-91. Editor(s): Roth, Juergen; Rutishauser, Urs; Troy, Frederick A., II. Birkhaeuser: Basel, Switz.
CODEN: 59FNAM
DOCUMENT TYPE: Conference
LANGUAGE: English

AB A genetic system was developed to investigate the mol. mechanisms of .alpha.2,8-linked polysialic acid (PSA) capsule synthesis, translocation, and regulation in the neuroinvasive bacterium E. coli K1. The 12 to 14 genes required for these processes are located in a multigenic kps cluster at chromosome unit 64. The cluster is composed of a central group of biosynthetic neu genes (region 2) that are flanked on either side by region 1 or 3 kps genes encoding general functions for PSA regulation, assembly, and translocation. The **polysialyltransferase** (polyST) encoded by K1 neuS was sequenced and compared to its homolog in **K92** E. coli, which synthesizes PSA chains with alternating sialyl .alpha.2,8-2,9 linkages. The results indicate that polySTs are processive enzymes which catalyze sequential transsialylations from donor CMP-sialic acid mols. to the nonreducing end of nascent PSA chains. The authors propose that the polymerase functions in a complex that includes the region 2 gene product of neuE and region 1 and 3 gene products of kpsMTSCDE. NeuE appears to function in the initiation or termination of PSA synthesis and may interact with polyprenol, as suggested by a dolichol-like binding site located in its predicted C-terminal membrane-spanning domain. These conclusions are supported by phenotypic anal. of mutants with multiple defects in sialic acid synthesis, degrdn., and polymn.

L4 ANSWER 9 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 5
ACCESSION NUMBER: 92:455923 SCISEARCH
THE GENUINE ARTICLE: JF345
TITLE: HOMOLOGY AMONG ESCHERICHIA-COLI K1 AND **K92** **POLYSIALYLTRANSFERASES**
AUTHOR: VIMR E R (Reprint); BERGSTROM R; STEENBERGEN S M; BOULNOIS G; ROBERTS I
CORPORATE SOURCE: UNIV ILLINOIS, COLL VET MED, DEPT PATHOBIOL, URBANA, IL, 61801 (Reprint); UNIV LEICESTER, DEPT MICROBIOL, LEICESTER LE1 9HN, ENGLAND
COUNTRY OF AUTHOR: USA; ENGLAND
SOURCE: JOURNAL OF BACTERIOLOGY, (AUG 1992) Vol. 174, No. 15, pp. 5127-5131.
ISSN: 0021-9193.
DOCUMENT TYPE: Note; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 31

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The neuS-encoded **polysialyltransferase** (polyST) in Escherichia coli K1 catalyzes synthesis of polysialic acid homopolymers composed of unbranched sialyl-alpha-2,8 linkages. Subcloning and complementation experiments showed that the K1 neuS was functionally interchangeable with the neuS from E. coli **K92** (S. M. Steenbergen, T. J. Wrona, and E. R. Vimr, J. Bacteriol. 174:1099-1108, 1992), which synthesizes polysialic acid capsules with alternating sialyl-alpha-2,8-2,9 linkages. To better understand the relationship between these polySTs, the complete **K92** neuS sequence was determined. The results demonstrated that K1 and **K92** neuS genes are homologous and indicated that the **K92** copy may have evolved from its K1 homolog. Both K1 and **K92** structural genes comprised 1,227 bp. There were 156 (12.7%) differences between the two sequences; among these mutations, 55 did not affect the derived primary structure of

K92 polyST and hence were synonymous with the K1 sequence. Assuming maximum simony, another estimated 17 synonymous mutations plus 84 nonsynonymous mutations could account for the 70 amino acid replacements in **K92** polyST; 36 of these replacements were judged to be conservative when compared with those of K1 polyST. There were no changes detected in the first 146 5' or last 129 3' bp of either gene, suggesting, in addition to the observed mutational differences, the possibility of a past recombination event between neuS loci of two different kps clusters. The results indicate that relatively few amino acid changes can account for the evolution of a glycosyltransferase with novel linkage specificity.

L4 ANSWER 10 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 6
ACCESSION NUMBER: 92:113032 SCISEARCH
THE GENUINE ARTICLE: HD467
TITLE: FUNCTIONAL-ANALYSIS OF THE SIALYLTRANSFERASE COMPLEXES IN ESCHERICHIA-COLI K1 AND K92
AUTHOR: STEENBERGEN S M; WRONA T J; VIMR E R (Reprint)
CORPORATE SOURCE: UNIV ILLINOIS, COLL VET MED, DEPT PATHOBIOL, URBANA, IL, 61801
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BACTERIOLOGY, (FEB 1992) Vol. 174, No. 4, pp. 1099-1108.
ISSN: 0021-9193.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 38

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The **polysialyltransferase** (polyST) structural gene, neuS, for poly-alpha-2,8sialic acid (PSA) capsule synthesis in Escherichia coli K1 was previously mapped near the kps region 1 and 2 junction (S. M. Steenbergen and E. R. Vimr, Mol. Microbiol. 4:603-611, 1990). Present Southern and colony blot hybrization results confirmed that neuS was a region 2 locus and indicated apparent homology with neuS from E. coli **K92**, bacteria that synthesize a sialyl-alpha-2,8-2,9-linked polymer. A K1- mutant with an insertion mutation in neuS was complemented in trans by **K92** neuS, providing direct evidence that neuS encoded the PSA polymerase. A 2.9-kb E. coli K1 kps subclone was sequenced to better characterize polyST. In addition to neuS, the results identified a new open reading frame, designated neuE, the linker sequence between regions 1 and 2, and the last gene of region 1, kpsS. The kpsS translational reading frame was confirmed by sequencing across the junction of a kpsS'-lacZ+ fusion. PolyST was identified by maxicell analysis of nested deletions and coupled in vitro transcription-translation assays. PolyST's derived primary structure predicted a 47,500-Da basic polypeptide without extensive similarity to other known proteins. PolyST activity was increased 31-fold and was membrane localized when neuS was cloned into an inducible expression vector, suggesting, together with the polyST primary structure, that polyST is a peripheral inner membrane glycosyltransferase. However, polyST could not initiate de novo PSA synthesis, indicating a functional requirement for other kps gene products. The existence of a sialyltransferase distinct from polyST was suggested by identification of a potential polyprenyl-binding motif in a C-terminal membrane-spanning domain of the predicted neuE gene product. Direct evidence for a quantitatively minor sialyltransferase activity, which could function to initiate PSA synthesis, was obtained by phenotypic analysis of mutants with multiple defects in sialic acid synthesis, degradation, and polymerization. The results provide an initial molecular description of K1 and **K92** sialyltransferase complexes and suggest a possible common function for accessory kps gene products.

L4 ANSWER 11 OF 11 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 19[redacted]380977 BIOSIS
DOCUMENT NUMBER: BR[redacted]47927
TITLE: SEQUENCE AND STRUCTURAL HOMOLOGY BETWEEN ESCHERICHIA-COLI K1 AND **K92 POLYSIALYLTRANSFERASES**.
AUTHOR(S): STEENBERGEN S; BERGSTROM R; VIMR E
CORPORATE SOURCE: UNIV. ILL., URBANA, ILL.
SOURCE: 92ND GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, NEW ORLEANS, LOUISIANA, USA, MAY 26-30, 1992.

ABSTR GEN MEET AM SOC MICROBIOL, (1992) 92 (0), 134.
CODEN: AGMME8.
DOCUMENT TYPE: Conference
FILE SEGMENT: BR; OLD
LANGUAGE: English